

Additional Information on the Morphology of *Potamotrygonocestus magdalenensis* (Tetraphyllidea: Onchobothriidae) from the Freshwater Stingray *Potamotrygon magdalena* in Colombia

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ABSTRACT: New specimens of *Potamotrygonocestus magdalenensis* were collected from *Potamotrygon magdalena* in the Ciénega Grande near Repelón, Colombia. The description of *P. magdalenensis* is emended to include information obtained from these specimens. Scanning electron microscopy revealed blade-like microtriches on the proximal and distal bothridial surfaces as well as on the neck and strobila. The blade-like microtriches of the neck and strobila are much more sparsely arranged than those on the scolex. The former microtriches are approximately twice as large as those on the proximal bothridial surfaces. The microtriches of the proximal bothridial surfaces are approximately 2–3 times as large as those on the distal bothridial surfaces. Filiform microtriches were visible among the blade-like microtriches of the neck and strobila. Spiniform microtriches cover the distal regions of the cirrus but are less numerous on its proximal regions. The inflated base of the cirrus is entirely devoid of microtriches. Cross-sections suggest that, in its nonfused regions, the ovary is essentially bilobed. Comparison of these new specimens with type material confirmed that several details in the original description by Brooks et al. (1976) require correction. The neck microtriches are much larger than those figured by Brooks et al. (1976). The vas deferens extends from the middle of the segment to the posterior end rather than from the posterior third, and the cirrus sac overlaps the posterior region of the ovary slightly in most segments, rather than being separated from it by more than 1 length. This species shares a number of similarities with *Calliobothrium pritchardae* and *Pedibothrium* species. Stingrays of a wide range of sizes were found infected with *P. magdalenensis*. Nymphs of an unidentified burrowing mayfly species were the only food items found in the stomachs of the small stingrays, suggesting that these insects are possible intermediate hosts for this cestode species.

KEY WORDS: *Potamotrygonocestus magdalenensis*, Cestoda, morphology, SEM.

As part of a broader study of the phylogenetic relationships of the Onchobothriidae, fresh material of *Potamotrygonocestus magdalenensis* Brooks and Thorson, 1976, was collected to facilitate description of this species with scanning electron microscopy (SEM). This paper details the results of the SEM. These collections also allowed us to provide new data on several aspects of the internal anatomy of this cestode species as well as new data on the prevalence and intensity of this species in the freshwater stingray *Potamotrygon magdalena* (Valenciennes, 1865).

Materials and Methods

A scientific collecting permit was obtained from INDERENA in Bogotá, through Eduardo Del Real Martínez, Subgerente de Pesca y Fauna Terrestre. All stingrays were collected near the Estación Piscícola Repelón field station on the Ciénega Grande near Repelón, Colombia, in May 1989. Spiral intestines of a total of 34 individuals of *Potamotrygon magdalena* were examined. The host individuals included 15 small stingrays (8–11 cm in disk width) and 19 large stingrays (19.6–29.8 cm in disk width). Six female and 8 male small individuals and 2 female and 17 male large individuals were necropsied. Small stingrays were collected using throw nets in the creek directly adjacent

to the field station. The presence of the remnant of the embryonic yolk sac in the smaller stingrays indicated that these individuals were very young. Large stingrays were purchased from fishermen using gill nets in the Ciénega Grande. The presence of embryos in most of the large females examined indicated that these hosts were sexually mature.

Ten specimens of *Potamotrygonocestus magdalenensis* were prepared as whole mounts for identification purposes. These specimens were stained in Harris' hematoxylin, dehydrated in ethanol, cleared in xylene, and mounted in Canada balsam. Twenty free segments were embedded in paraplast (Sherwood Medical Industries, St. Louis, Missouri), and serial cross-sections were cut at 10-μm intervals with an American Optics rotary microtome. Sections were floated on water on slides coated with egg albumin, air-dried, stained in Gill's hematoxylin, counterstained with eosin, dehydrated in an ethanol series, cleared in xylene, and mounted in Canada balsam. Ten scoleces and 10 free segments were prepared for SEM. These specimens were hydrated in an ethanol series, treated with 1% osmium tetroxide overnight, dehydrated in an ethanol series, and air-dried using Peldri II (Ted Pella, Inc., Redding, California) according to the procedure given by Freidenfelds et al. (1994). Dried specimens were mounted directly on stubs using silver paint, sputter-coated with gold for 1 min (approximately 100 Å), and examined with a Coates and Welter field emission scanning electron microscope.

Illustrations were drawn with the aid of a drawing tube. All measurements are in micrometers unless otherwise stated.

Results

Two of the 15 small stingrays and 17 of the 19 large stingrays examined were infected with *P. magdalenensis*. Each of the small stingrays was infected with 2 individuals. The mean intensity of infection for the large stingrays was 5.4 ± 8.2 with a range of 1–35 individuals per host. All specimens were found in the anterior portion of the spiral intestine; the exact chamber of origin was not determined.

Potamotrygonocestus magdalenensis

Brooks and Thorson, 1976
(Figs. 1–10)

The following information emends the description of Brooks and Thorson (1976) for this species. Proximal surfaces of bothridia covered with closely spaced, long, blade-like microtriches approximately 2–3 μm in length, which taper to a point (Fig. 2). Distal surfaces of bothridia densely covered with small, spiniform microtriches, approximately 1 μm in length (Fig. 3). Neck covered with widely spaced, large, blade-like microtriches (Fig. 4), approximately 8 μm in length, characterized by longitudinal striae, interspersed with numerous, small, filiform microtriches (Fig. 4). Cephalic peduncle lacking.

Distal portion of cirrus covered with narrow, spiniform microtriches, approximately 2–3 μm in length (Figs. 6, 7); microtriches more sparsely distributed proximally. Base of cirrus characterized by inflated bulb, lacking microtriches (Fig. 6). Vas deferens extending from middle of segment (Fig. 8) to terminal genital pore. Ovary bilobed in cross-section (Fig. 9); lobes fused at ovarian bridge and at posterior extremity. Eggs single, lacking filaments. Vitellaria in single lateral bands. Segments covered with numerous, short, densely arranged microtriches interspersed with large blade-like microtriches, approximately 8 μm in length (Fig. 5).

TYPE AND ONLY KNOWN HOST: *Potamotrygon magdalenae* (Valenciennes, 1865).

SITE OF INFECTION: Spiral intestine.

TYPE LOCALITY: Ciénega Rabón, vicinity of San Cristóbal, Bolívar, Colombia.

ADDITIONAL LOCALITY: Ciénega Grande, Estación Piscícola Repelón (new record).

MATERIAL EXAMINED: Paratypes: USNM

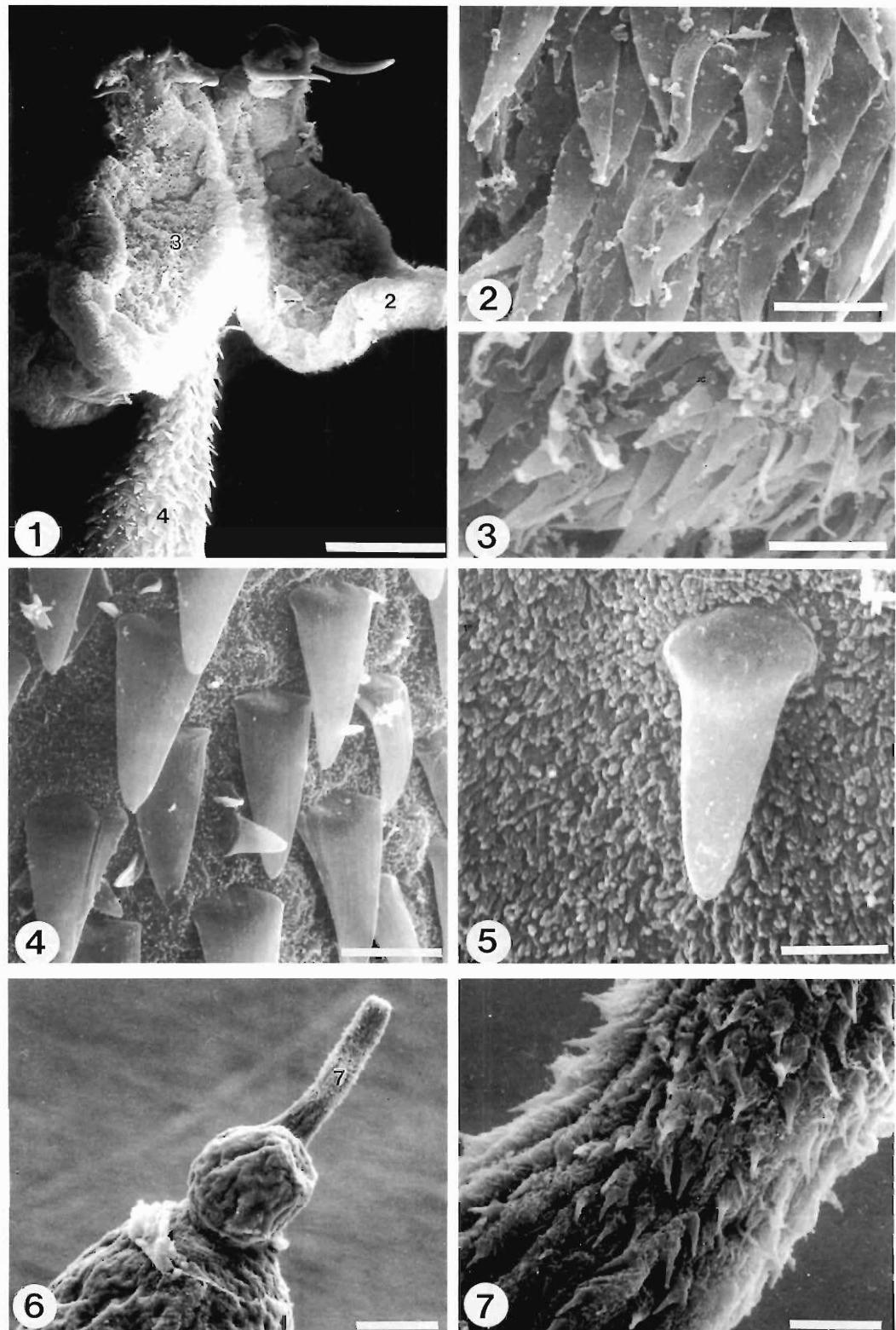
Helm. Coll. No. 73543; Univ. Neb. State Mus. H. W. Manter Lab. No. 20254. One whole mount from new locality and material illustrated in Figures 8–10 deposited at HWML (No. 37546); additional specimens retained in senior author's personal collection.

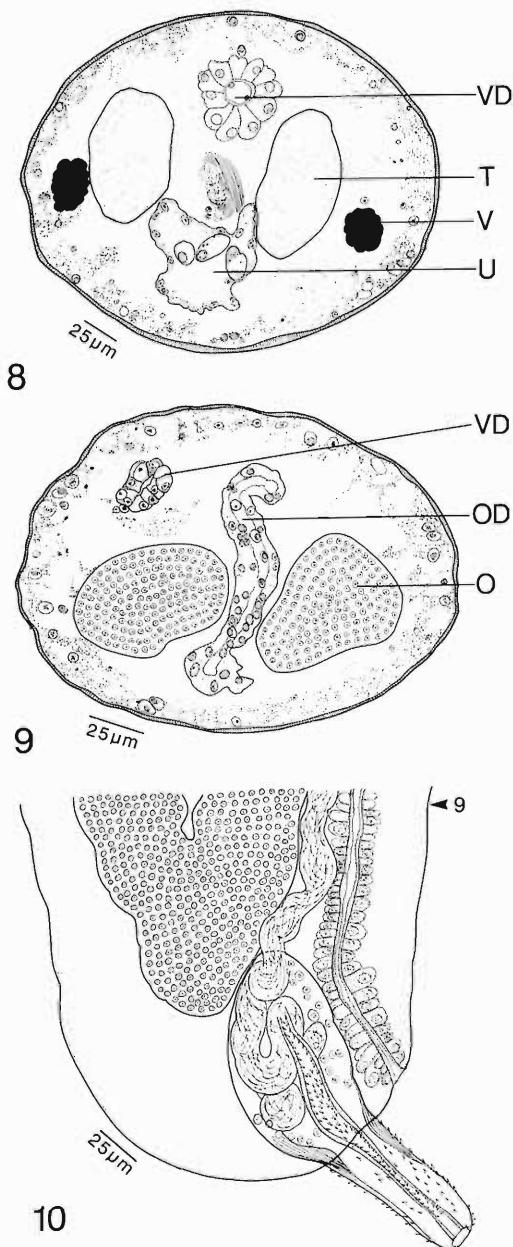
Discussion

SEM reveals a number of similarities between the patterns of microtriches on the scolex of *Potamotrygonocestus magdalenensis* and species of *Pedibothrium* Linton, 1909, and *Calliobothrium pritchardae* Caira and Ruhnke, 1990. In all of these species, the proximal and distal bothridial surfaces exhibit blade-like microtriches, which differ somewhat in shape between the 2 surfaces. In *P. magdalenensis*, the microtriches also differ in size, with those on the proximal surfaces being much larger than those on the distal bothridial surfaces. Unlike *Pedibothrium* species, *P. magdalenensis* has no distinct scolex peduncle; microtriches are found on all external surfaces of the scolex and strobila. Our cross-sections of the ovary of *P. magdalenensis* indicate that the ovary is essentially bilobed. Its overall morphology is strikingly similar to that of *C. pritchardae*: both are inverted A-shaped, and both are basically bilobed in cross-section. This is very different from the condition of the ovary found in *Pedibothrium* species.

Our work indicates that several features of this species were incorrectly described by Brooks and Thorson (1976). The microtriches of the neck are much larger than those shown in the scolex illustration of this species presented by these authors (compare our Fig. 1 to their Fig. 1). The cirrus sac, at least in free segments, is not separated from the ovary as figured by Brooks and Thorson (1976) but overlaps slightly with the posterior region of the ovary, similar to the condition figured for *Potamotrygonocestus amazonensis* Mayes, Brooks, and Thorson, 1981, by Mayes et al. (1981) (see their Fig. 10). In all of the free segments of *P. magdalenensis* we examined, the vas deferens extended from the middle of the segment, rather than the posterior third as figured by Brooks and Thorson (1976). This explains why our section through the testes in the middle of the segment contained a section through the vas deferens (Fig. 8).

Our data indicate that this parasite is able to infect very young stingrays. We found adult specimens of *Potamotrygonocestus magdalenensis* in





2 stingrays with disk widths of only 9 and 9.3 cm, respectively; both stingrays still retained fairly large remnants of their embryonic yolk sacs. This size is very similar to the size of neonatal individuals of this species; one of the pups we removed from a pregnant female had a disk width of 7.5 cm. Preliminary examination of the stomach contents of the small stingrays suggests that these animals feed exclusively on nymphs of an unidentified burrowing mayfly species. This invertebrate would be a good place to begin the search for intermediate hosts of *P. magdalenensis*.

Acknowledgments

We are very grateful to Oscar Valencia, Director of the Estación Piscícola Repelón, for allowing us to use the facilities at the station and for making local arrangements for collection of stingrays. The trip to Colombia would not have been possible without the capable assistance of Marta Martinez-Wells, who translated all of our correspondence with Colombian officials prior to the trip and acted as translator and assisted with dissections in the field. We are indebted to Edwardo Velasco, who traveled to Bogata to facilitate the issuing of our permits. We also thank Kelly Steele for assisting with ray dissections despite her botanical inclinations and 2 anonymous reviewers for their helpful comments on an ear-

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Figures 8–10. Line drawings of *Potamotrygonocestus magdalenensis*. 8. Cross-section through testes in middle of segment (voucher specimen, HWML No. 37546); note 3 eggs in uterus. 9. Cross-section through ovary posterior to ovarian bridge and anterior to fused posterior lobes of ovary (voucher specimen, HWML No. 37546). 10. Detail of terminal genitalia from whole mount of free segment (HWML No. 37546). Arrow indicates location of section through ovary shown in Figure 9. Abbreviations: O = ovary, OD = oviduct, T = testis, U = uterus, V = vitellaria, VD = vas deferens.

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Figures 1–7. Scanning electron microscopy of *Potamotrygonocestus magdalenensis*. 1. Scolex. Locations at which Figures 2–4 were taken are indicated with corresponding numbers. 2. Microtriches on proximal bothridial surface. 3. Microtriches on distal bothridial surface. 4. Microtriches on neck. 5. Microtriches on mature segment. 6. Everted cirrus. Number indicates location at which Figure 7 was taken. 7. Enlarged view of cirrus microtriches. Scale bar in Figure 1 = 40 μm; scale bar in Figures 2, 3, and 5, = 2 μm; scale bar in Figure 4 = 4 μm; scale bar in Figure 6 = 50 μm; scale bar in Figure 7 = 5 μm.

lier version of this manuscript. This work was supported in part by grant IBN-9007613 from the National Science Foundation to J.N.C.

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Early History of USDA Livestock Parasitology Available

Animal Parasite Research in the Zoological Division, Bureau of Animal Industry, U.S. Department of Agriculture, Washington, D.C., 1923-1938, by John S. Andrews, is a history of classical research on livestock parasites and of the development of parasitology research facilities in Beltsville, Maryland. Copies of this book are available without cost, except for \$4.00 for packaging and postage, from Dr. John S. Andrews, Jr., 4210 Quail Ridge Way, Norcross, GA 30092.